

WHAT IS CLAIMED IS:

1. A method of producing high titer of packaged alphavirus particles, comprising the steps of:

5 (a) transfecting a first set of cells with (i) a alphavirus replicon; (ii) a first helper RNA comprising a sequence encoding an alphavirus capsid protein and cis-acting elements that allow efficient replication of said first helper RNA and (iii) a second helper RNA comprising a sequence encoding the alphavirus 10 glycoproteins E1 and E2 and cis-acting elements that allow efficient replication of said second helper RNA;

(b) obtaining a primary stock of viral particles comprising (i) viral particles containing said alphavirus replicons, (ii) viral particles containing said first helper RNA comprising a 15 sequence encoding an alphavirus capsid protein, and (iii) viral particles containing said second helper RNA comprising a sequence encoding the alphavirus glycoproteins E1 and E2;

(c) infecting a second group of cells with said primary stock of viral particles at high multiplicity of infection; and

20 (d) obtaining high titers of secondary stock of packaged viral particles comprising (i) viral particles containing said

alphavirus replicons, (ii) viral particles containing said first helper RNA comprising a sequence encoding an alphavirus capsid protein, and (iii) viral particles containing said second helper RNA comprising a sequence encoding the alphavirus glycoproteins E1 and

5 E2.

2. The method of claim 1, further comprising the steps of:

10 (a) infecting said second group of cells with said secondary stock of viral particles at a high multiplicity of infection; and

(b) obtaining high titers of viral particles comprising (i) viral particles containing said alphavirus replicons,

15 (ii) viral particles containing said first helper RNA comprising a sequence encoding an alphavirus capsid protein, and (iii) viral particles containing said second helper RNA comprising a sequence encoding the alphavirus glycoproteins E1 and E2.

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3. The method of claim 1, wherein said alphavirus replicon and said first and second helper RNAs are delivered to the cells in plasmid form or in RNA form.

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4. The method of claim 1, wherein said alphavirus replicon is a Sindbis virus replicon.

5. The method of claim 1, wherein said capsid protein 10 and glycoproteins are from an alphavirus selected from the group consisting of Sindbis virus, Venezuelan Equine encephalitis virus, Ross River virus, and Semliki Forest virus.

15 6. The method of claim 1, wherein said cis-acting elements comprise tRNA<sup>Asp</sup> and replicational enhancer of Sindbis virus.

7. The method of claim 1, wherein said primary stock of viral particles have a titer of at least  $5 \times 10^8$  infectious units per ml of media.

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8. A method of generating a high titer of packaged alphavirus particles for large-scale production of recombinant protein, comprising the steps of:

(a) transfecting a first group of cells with (i) an alphavirus replicon comprising a sequence encoding a heterologous protein; (ii) a first helper RNA comprising a sequence encoding an alphavirus capsid protein and cis-acting elements that allow efficient replication of said first helper RNA and (iii) a second helper RNA comprising a sequence encoding the alphavirus glycoproteins E1 and E2 and cis-acting elements that allow efficient replication of said second helper RNA;

(b) obtaining a primary stock of viral particles comprising (i) viral particles containing said alphavirus replicons comprising a sequence encoding said heterologous protein, (ii) viral particles containing said first helper RNA comprising a sequence encoding an alphavirus capsid protein, and (iii) viral particles

containing said second helper RNA comprising a sequence encoding the alphavirus glycoproteins E1 and E2;

(c) infecting a second group of cells with said primary stock of viral particles at high multiplicity of infection;

5 (d) obtaining high titers of a secondary stock of viral particles comprising (i) viral particles containing said alphavirus replicons comprising a sequence encoding said heterologous protein, (ii) viral particles containing said first helper RNA comprising a sequence encoding an alphavirus capsid protein, 10 and (iii) viral particles containing said second helper RNA comprising a sequence encoding the alphavirus glycoproteins E1 and E2; and

(e) infecting host cells with the viral particles of

(d) in a large scale production of said heterologous protein encoded 15 by said viral particles.

9. The method of claim 8, wherein said host cells are mammalian cells or insect cells.

10. The method of claim 8, wherein said alphavirus replicon and said first and second helper RNAs are delivered to the cells in plasmid form or in RNA form.

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11. The method of claim 8, wherein said alphavirus replicon is a Sindbis virus replicon.

10 12. The method of claim 8, wherein said capsid protein and glycoproteins are from an alphavirus selected from the group consisting of Sindbis virus, Venezuelan Equine encephalitis virus, Ross River virus, and Semliki Forest virus.

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13. The method of claim 8, wherein said cis-acting elements comprise tRNA<sup>Asp</sup> and replicational enhancer of Sindbis virus.

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14. The method of claim 8, wherein said secondary stock of viral particles have a titer of at least  $5 \times 10^8$  infectious units per ml of media.